厚生労働科学研究費補助金(化学物質リスク研究事業)
「化学物質リスク評価法の国際的バリデーションに関する研究 (H18-化学-一般-003)」

総合研究報告書

OECD 活動と国際協調

研究分担者　小島　肇　国立医薬品食品衛生研究所・薬理部

研究要旨

2007 年および 2008 年に OECD/ Endocrine Disruption Testing and Assessment Task Force (EDTA) Validation Management Team-Non Animal (VMT-NA) 会議に参加し、現在進めている国際バリデーション研究の進捗について意見交換した。2008 年 10 月にベルリンで開催された皮膚刺激性専門家会議に参加し、新たなテストガイドライン案について意見交換するとともに、日本のバリデーション結果を報告した。

また、OECD ガイドライン化を目指し、本研究班の成果に基づき、内分泌かく乱性スクリーニング法である Lumi-cell 法については米国より、HeLa 細胞をベースにしたエストロジェン受容体αに対するレポーターアッセイ試験法 (HeLa 法) については経済産業省より、遺伝毒性試験であるコメットアッセイ、皮膚感作性試験 Local Lymph Node Assay (LLNA) の変法である非放射線物質による LLNA 法および培養表皮モデル LabCyte EPI-MODEL24 を用いた皮膚刺激性試験については、Standard Project Submission Form (SPSF) が厚生労働省のナショナルコーディネーターより OECD に提出された。その中で、HeLa 法のアゴニストアッセイについては 2009 年 4 月、OECD ガイドライン 455 としての認証を受けた。

A. 研究目的

動物実験代替法に関しては、化粧品の安全性評価法を中心に、多くの検討が行われている。皮膚腐食性試験や光毒性試験代替法などにおいては、欧米および我が国において大規模なバリデーション研究と専門家による第三者評価（以下、第三者評価と記す）が実施され、一部がOECDのガイドラインに取り入れられ、化学物質の評価にも用いられている。しかし、感作性試験や生殖毒性試験など、まだ開発やOECD基準に則ったバリデーション研究がなされていないものも多い。一方、内分泌かく乱化学物質の*in vitro*評価法については無細胞系受容体結合試験、酵母等各種導入受容体結合試験、各種受容体導入レポーター遺伝子転写活性化試験（Lumi-cell法など）、化学物質評価研究機構（CERI）が開発したHeLa細胞をベースにしたエストロジェン受容体αに対するレポーターアッセイ試験法の他、アロマターゼ活性化試験など、いくつかの方法が開発され、OECD基準に則ったバリデーション研究が行われている。DNA損傷性を調べるコメットアッセイについても、*in vitro*および*in vivo*の試験法が開発されているが、データの評価、解釈のみならず方法論に関しても未熟であり、国際的なガイドラインは作成されていない。

本研究班はこれら今まで評価が遅れていた化学物質の安全性評価のための試験法をOECDの基準に則ってバリデーション研究と第三者評価を行うものである。また、極めて多大な労力を有し、大学や個々の研究機関、更には、一国では実施困難な多施設バリデーション研究と第三者評価を国際的な協力のもとで実施し、本研究で検討した試験方法のOECDガイドライン化を目指すものである。そこで、我々の開発した方法を将来、OECDの試験法ガイドライン化するための活動を行うとともに、関連するOECD活動に協力した。

本報告書では、日本で開発あるいは日本が中心となって開発している方法を中心に、OECDにおける活動および国際協調をまとめた。

B. 研究方法および結果

B-1 OECD/EDTA VMT-NAでの会合

2007年11月13日～15日にイタリア イスプラで開催された OECD/ Endocrine Disruption Testing and Assessment Task Force (EDTA) Validation Management Team-Non Animal (VMT-NA) 会議に日本から小野 敦博士、小島 肇（以上、国立医薬品食品衛生研究所）、武吉正博博士、赤堀有美博士が出席した。各種の内分泌かく乱物質スクリーニングの現状を確認するとともに、日本からも共同研究内容について種々の提案を行った。

OECD VMT-NA で検討が進められている各種の内分泌かく乱物質スクリーニングの進捗について報告があり、各国の代表とその内容について意見交換した。特に、日本で開発されたHeLa細胞をベースにしたエストロジェン受容体αに対するレポーターアッセイについては、OECDガイドライン成立を目指し、日本主導でバリデーションの準備を進めていると報告し、その内容に助言を頂いた。

2008年11月19日～21日にフランス パリOECD本部で開催された OECD/EDTA VMT-NA 会議には、日本から小野 敦博士、小島 肇（以上、国立医薬品食品衛生研究所）、武吉正博博士（化学物質評価研究機構）が出席した。OECD/EDTA VMT-NAで検討が進められている各種の内分泌かく乱物質スクリーニングの進捗について報告があり、各国の代表とその内容について意見交換した。

日本で進捗中のバリデーション研究の内容について報告した。特に、日本で開発されたHeLa細胞をベースにしたエストロジェン受容体αに対するレポーターアッセイの進捗について報告し、その内容に助言を得た。

B-2 皮膚刺激性専門家会議

2008年10月21日～22日にベルリン BfR (Federal Institute for Risk Assessment) で開催された皮膚刺激性専門家会議に小島 肇（国立医薬品食品衛生研究所）が参加した。

EUより提案のあった培養表皮モデルを用いた皮膚刺激性試験テストガイドライン案について意見交換するとともに、培養表皮モデル LabCyte EPI-MODEL24 を用いた皮膚刺激性試験に関する日本のバリデーション結果を報告し、その内容を今後考慮するように求めた。

B-3 眼刺激性試験専門家会議

2008年12月4日～5日にワシントンD.C.で開催された眼刺激性専門家会議には日本から参加しなかった。

しかし、米国より提案のあった摘出牛角膜試験および摘出鶏眼球試験テストガイドライン案について事前に意見を送った。

B-4 SPSFの提出

我々の研究成果を踏まえ、2008年には、内分泌かく乱性スクリーニング法であるLumi-cell法については米国より、HeLa細胞をベースにしたエストロジェン受容体αに対するレポーターアッセイ試験法（HeLa法）については経済産業省より、遺伝毒性試験であるコメットアッセイについては厚生労働省より、試験法の予備登録である Standard Project Submission Form (SPSF) が OECD に提出された。

2009年には、皮膚感作性試験 Local Lymph Node Assay (LLNA) の変法である非放射線物質によるLLNAおよび培養表皮モデル LabCyte EPI-MODEL24 を用いた皮膚刺激性試験の OECD ガイドライン化を目指し、Standard Project Submission Form (SPSF) が2009年1月に厚生労働省より OECD に提出された。

その中で、HeLa 法のアゴニストアッセイについ

ては2009年4月、OECDガイドライン455としての認証を受けた。

C. 考察

本研究班のテーマである「化学物質リスク評価法の国際的バリデーション」の目的は、安全性評価に有用な新規試験法を公定化することである。その最終的な目標がOECDガイドラインの確立であることから、これを目指してSPSFを提出し、本研究班のバリデーション結果から国際的検討に至るよう努力するものである。

なお、OECDガイドラインとして完成させるまでには、短くても3年、通常5年以上掛かることを銘記しなければならない。この点でより迅速な国際合意を図るために共同研究は重要と考えている。

E. 結論

OECDの新たなガイドラインの成立に協力するとともに、日本からもSPSFを提出して積極的な試験法の開発を進めている。その中で、HeLa法については2009年4月、OECDガイドライン455としての認証を受けた。

F. 健康危険情報

なし

G. 知的財産権の出願・登録状況

なし

H. 研究発表

1．論文発表

1) 小島肇夫：動物実験代替に関する最近の動向、化粧品技術者会誌、40(4)263-268 (2006)

2) 小島肇夫：JaCVAMの設立と使命、日皮協ジャーナル、57、129 (2007)

3) 小島肇夫：バリデーションについて、最新動物実験代替法、技術情報協会 pp. 267-273 (2007)

4) 小島肇夫：日本における動物実験代替法の開発動向、Fragrance Journal 10、29-34 (2007)

5) 小島肇夫：動物実験代替法のバリデーション、COSMETIC STAGE, 8、54-56 (2007)

6) 小島肇夫：代替法国際動向から見た新技術導入の可能性、動物実験代替法のためのバイオマテリアル・デバイス、シーエムシー出版 pp. 1-5 (2007)

7) 小島肇夫：動物実験代替法の現状と展望、日本薬理学会会誌、130、505-509 (2008)

8) Kojima, H.: JaCVAM: An organization supporting the validation and peer review of new alternatives to animal testing, WC6 proceedings, AATEX, special issue, 483-485 (2008)

9) 小島肇夫：EUにおける動物実験代替法の現況とREACH対策、日皮協ジャーナル、30(2) 156-162 (2008)

10) Kojima, H.: Perspectives on validation and regulatory acceptance of animal alternative testings in Japan, P&G Actives Risk Communication, 2(1), 1-4 (2008)

11) 小島肇夫：in vivo経皮吸収試験法、最新・経皮吸収剤～開発と基礎から申請のポイントまで～、株式会社情報機構、東京、pp. 95-103 (2008)

12) 小島肇夫：in vitro経皮吸収試験法、最新・経皮吸収剤～開発と基礎から申請のポイントまで～、株式会社情報機構、東京、pp. 104-113 (2008)

13) 小島肇夫：安全性評価と動物実験代替法の現状、薬学雑誌、128 (5) 747-752. (2008)

14) 小島肇夫：動物実験の3Rsにおける国内外の動向、ファルマシア、44(9)、857-861 (2008)

15) 小島肇夫：REACH対応に必要な動物実験代替法の現状、コスメティックステージ、2 (5)、1-4 (2008)

16) 小島肇夫：動物実験代替法に関する2008年の国際動向、Fragrance Journal、2009-1、65-69 (2009)

17) 小島肇夫：動物実験代替法の現状と展望、J. Environ Dermatol Cutan Allergol, 3 (1)、1-6 (2009)

18) T Omori, T., Idehara, K., Kojima H., Sozu, T., Arima, K., Goto, H., Hanada, T., Ikarashi, Y., Inoda, T., Kanazawa, Y., Kosaka, T., Maki, E., Morimoto, T., Shinoda, S., Shinoda, N., Takeyoshi, M., Tanaka, M., Uratani, M., Usami, M., Yamanaka, A., Yoneda, T., Yoshimura, I., Yuasa, A.: Interlaboratory validation of the modified murine local lymph node assay based on adenosine triphosphate measurement, Journal of Pharmacological and Toxicological Methods 58 11-26 (2008)

19) Arai, S., Yamamoto, N., Kato M., and Kojima, H.: An *in vitro* evaluation methods to test ocular irriation using a human corneal epithelium model, *Altern. Animal Test. EXperiment*, 13(2), 83-90 (2008)

20) Kojima, H., Ando, A., Inagaki, K., Ohhira, M., Kosaka, T., Nakamura, Y., Torishima, H., Morikawa, N., Kanno, J., Kuboki, M., Genno, G., Nokata, M., Harada, T., Morimoto, T., Yoshimura, I. and Ohno, Y.: Validation of human skin models for skin corrosibvity tests in Japan, AATEX 13, 36-44 (2008)

21) 小島肇夫：動物実験の3Rsにおける国内外の動向、城西大学生命科学研究センター報告第7号、p37-50 (2009)

2．学会発表

1) Kojima, H.: Current activities on alternative research in Japan, KSOT/KEMS Spring Annual Meeting, Alternative Toxicology and Marine Ecotoxicology, Korea (2006)
2) 小島　肇：JaCVAM (Japanese Center for the Validation of Alternative Methds) 新規試験法評価室の紹介、日本環境変異原学会　MMS 研究会第 49 回定例会、熱川 (2006)
3) Kojima, H.: JaCVAM Update, Scientific Advisory Committee on Alternative Toxicological Methods, North Carolina (2006)
4) 小島　肇：JaCVAM の設立と使命、日本産業皮膚衛生協会第 39 回研修会、京都 (2006)
5) 小島　肇：特別企画 1 ：動物実験代替法に関する最近の国内外の動向、国内において現在進行中の評価試験プロジェクト紹介、日本動物実験代替法学会第 20 回大会、東京 (2006)
6) 小島　肇：動物実験代替法開発の推進とその評価：JaCVAM の設立と役割、日本薬学会第 127 年会、富山 (2007)
7) Kojima, H.: JaCVAM Update, Scientific Advisory Committee on Alternative Toxicological Methods, Bethesda, (June, 2007)
8) Kojima, H.: JaCVAM Update, 6th World Congress on Alternatives 6 Animal Use in the Life Sciences, p. 119, Tokyo (2007)
9) Kojima, H.: JaCVAM Process to Validate and Peer Review of New Alternative Methods, 6th World Congress on Alternatives 6 Animal Use in the Life Sciences, p. 82, Tokyo (2007)
10) Kojima, H.: Validation study using Japanese models, 6th World Congress on Alternatives 6 Animal Use in the Life Sciences, p. 64, Tokyo (2007)
11) Kojima, H.: JaCVAM Update, ECVAM Scientific Advisory Committee, Ispra (November , 2007)
12) 小島　肇：代替法を取り巻く内外の動きと今後の方向性、皮膚基礎研究クラスターフォーラム第 2 回教育セミナー、東京 (2007)
13) 小島　肇：動物実験と代替法の現状、城西大学生命科学研究センター講演会、城西大学 (2007)
14) 小島　肇：EU における動物実験代替法の現況と REACH 対策、日皮協・会員研究会、京都 (2007)
15) Arai, S., Saitou, M., Takashima, Y. Honma, M. and Kojima, H.: Anew trial for in vitro Comet assay using a 3-dimensional human epidermal model, 36th Annual Meetings of the Japanese Environmental Mutagen Society, Kitakyusu (2007)
16) Kojima, H., et al: Validation and implementation, Round table on International aspects of validation & accept, Regulatory Acceptance and Implementation of 3R approach, Annual Conference 2007 EPAA, Brussels (2007)
17) Kojima, H.: The Importance of the in vivo comet assay in genotoxicity testing, Predictive Human Toxicity and ADME/TOX studies, 3rd Annual conference of Mondial Research Presentation, Brussels (2008)
18) Kojima, H., et al.: Panel Discussion, ICCVAM Ten-Year Anniversary Symposium, Washington DC (2008)
19) 小島　肇：日本の動向と JaCVAM の活動、JaCVAM 第 1 回ワークショップ「昨今の試験法ガイドラインを巡る国際動向」、東京 (2008)
20) Kojima, H.: Report JaCVAM, ECVAM Scientific Advisory Committee, Ispra (May, 2008)
21) 小島肇夫：REACH 対応と動物実験代替法、第 128 回 FJ セミナー、東京 (2008)
22) Kojima, H.: Japanese Collaboration on Alternative to Animal Toxicology Testing, World Congress on in Vitro Biology, Tucson (2008)
23) Kojima, H.: JaCVAM Update, Scientific Advisory Committee on Alternative Toxicological Methods, Bethesda, (June, 2008)
24) 小島肇、武吉正博、大森崇、寒水孝司、有馬和範、出原賢治、金澤由基子、牧栄二、中桐直人、五十嵐良明、田中正志、吉村功、湯浅敦子：LLNA-BrdU 法の施設間バリデーション研究、第 35 回日本トキシコロジー学会学術年会、東京 (2008)
25) 小島肇、武吉正博、出原賢治：非 RI 法による皮膚感作性試験代替法 (LLNA 法) のバリデーション研究－試験法概要－、第 15 回日本免疫毒性学会学術大会、東京 (2008)
26) 出原賢治、小島肇、武吉正博：非 RI 法による LLNA 法の比較、第 38 回日本皮膚アレルギー・接触皮膚炎学会総会学術大会、大阪 (2008)
27) Kojima, H.: Report JaCVAM, ECVAM Scientific Advisory Committee, Brussels (November, 2008)
28) Kojima, H.: International Current of 3Rs International Symposium on the 3Rs Promotion in Asia, Saitama (2008)
29) 小島　肇：医薬部外品の製造販売承認申請における安全性に関する資料のあり方検討会報告、日本動物実験代替法学会第 21 回大会、埼玉 (2008)
30) 杉山真理子、河合敬一、小島　肇、寒水孝司、辰見寿、夏秋優、森福義：医薬部外品の製造販売承認申請における安全性に関わるあり方検討委員会報告　皮膚刺激性分科会からの報告、日本動物実験代替法学会第 21 回大会、埼玉 (2008)

31) 金澤由基子、横関博雄、中田土起丈、坂口斉、大野泰雄、小島　肇：医薬部外品の製造販売承認申請における安全性に関わるあり方検討委員会報告　感作性試験分科会からの報告、日本動物実験代替法学会第21回大会、埼玉（2008）

32) 瀬戸　洋一、萩野　滋延、畠　賢一郎、森田正道、平野　耕治、金子　豊蔵、小島　肇：医薬部外品の製造販売承認申請における安全性に関わるあり方検討委員会報告　眼刺激性分科会からの報告、日本動物実験代替法学会第21回大会、埼玉（2008）

33) 藤井まき子、小島　肇、杉林堅次、上月裕一、桑原裕史：医薬部外品の製造販売承認申請における安全性に関わるあり方検討委員会報告　皮膚透過・経皮吸収試験分科会からの報告、日本動物実験代替法学会第21回大会、埼玉（2008）

34) 笠松俊夫、江幡真也、林　真、能美建彦、本間正充、小島　肇：医薬部外品の製造販売承認申請における安全性に関わるあり方検討委員会報告　遺伝毒性分科会からの報告、日本動物実験代替法学会第21回大会、埼玉（2008）

35) 武吉正博、小島　肇、大森崇、寒水孝司、有馬和範、出原賢治、金澤由基子、牧栄二、中桐直人、五十嵐良明、田中正志、吉村功、湯浅敦子：LLNA-BrdU法の施設間バリデーション研究、日本動物実験代替法学会第21回大会、埼玉（2008）

36) 小島　肇、安藤洋子、山口能宏、小坂忠司、鈴木民恵、湯浅敦子、渡邉幸彦、篠田伸介、出原賢治、吉村　功、宮岡悦良、石山賢也、加藤雅一、大森　崇：培養皮膚モデル LabCyte EPI-MODEL24 を用いた皮膚刺激性試験代替法のバリデーション研究　予備試験結果、日本動物実験代替法学会第21回大会、埼玉（2008）

37) 小島　肇：in vitro 遺伝毒性試験－光と影－、第37回日本環境変異原学会、沖縄（2008）

38) 小島　肇：毒性試験における培養細胞の利用、安全性評価研究会２００８年冬のセミナー、東京(2008)

39) 小島　肇：動物実験適正化のグローバルな動き－代替法の動きを中心に－、日本制約工業協会医薬品評価委員会　第102回基礎研究部会総会、京都（2009）

40) Kojima, H. : Current aspects of LLNA-DA and LLNA-BrdU as alternatives for skin sensitizer classification in Japan, 2009 Winter Conference of Korean Society of Alternatives to Animal Experiments, Seoul (February, 2009)

41) Kojima, H. : Report JaCVAM, ECVAM Scientific Advisory Committee, Ispra (March, 2009)

42) Strickland, J., Paris, M., Allen, D., Tice, R., Kojima, H., Prieto, P., Wind, W., Stokes, W. : ICCVAM/NICEATM/ECVAM/JaCVAM Scientific Workshop on Acute Chemical Safety Testing: Advancing *In Vitro* Approaches and Humane Endpoints for Acute Systemic Toxicity Evaluations, 48th Annual SOT meeting, Baltimore (2008)

I. 添付資料

添付資料1：Draft Report of the 5th meeting of the validation management group for non-animal testing (vmg-na)

添付資料2：SPSF　Stably transfected Transcriptional Activation (TA) assay for detection of anti-estrogenic activity of chemicals

添付資料3：SPSF　Stably transfected Transcriptional Activation (TA) assay for detection of androgenic and anti-androgenic activity of chemicals

添付資料4：SPSF　IN vivo Comet Assay in Genotoxicity Testing

資料5：DRAFT REPORT OF THE OECD EXPERT CONSULTATION MEETING FOR THE REVISION OF THE DRAFT *IN VITRO* SKIN IRRITATION TEST GUIDELINE

資料6：発表資料

資料7：Draft Report of the 6th meeting of the validation management group for non-animal testing (vmg-na)

資料8：HeLa　ATG　VMG-NA6 発表原稿

資料9：OECD TEST GUIDELINES PROGRAMME, Standard Project Submission Form Non-Radioisotope version of the Local Lymph Node Assay (LLNA)

資料10：OECD TEST GUIDELINES PROGRAMME, Standard Project Submission Form *In vitro* human epidermal model to assess skin irritation: LabCyte EPI-MODEL24

添付資料11：DRAFT TEST GUIDELINE 455: THE STABLY TRANSFECTED HUMAN ESTROGEN RECEPTOR-α TRANSCRIPTIONAL ACTIVATION ASSAY FOR DETECTION OF ESTROGENIC AGONIST ACTIVITY OF CHEMICALS

Patric.Amcoff@oecd.org
Paris, 19 December 2007

DRAFT REPORT OF THE 5TH MEETING OF THE VALIDATION MANAGEMENT GROUP FOR NON-ANIMAL TESTING (VMG-NA)

13-15 November 2007, ECVAM-DG JRC, Ispra, Italy

INTRODUCTION

1. The 5th Meeting of the Validation Management Group for Non-Animal tests (VMG-NA) was held in Ispra, Italy on 13th-15th November 2007 at the European Center for the Validation of Alternative Methods (ECVAM) at the Joint Research Center (JRC). The main objective of the VMG-NA is to identify or propose validated or promising non-animal assays for endocrine chemicals testing, and develop and validate tools necessary for the Level 2 (*In vitro* assays providing mechanistic data) of the Conceptual Framework of the Endocrine Disruption Testing and assessment Task Force of the Test Guidelines Programme (EDTA), in addition to report the progress of ongoing co-operations and developments of new tests that was initiated at previous VMG-NA meetings.

2. The list of participants of the Meeting is attached to this report as Annex 1.

3. Patric Amcoff of the Secretariat opened the Meeting and welcomed participants of the VMG NA5 on behalf of the OECD Secretariat and acknowledged ECVAM for hosting the meeting. He explained OECD procedures and introduced Dr. Steve Bradbury (US EPA) and Dr. Daniel Dietrich (Konstanz University, Germany) as the co-chairs of the meeting.

ADOPTION OF THE DRAFT AGENDA

4. The Secretariat introduced the agenda and asked the meeting for some degree of flexibility since the time estimated for several of the agenda items were difficult to foresee. The agenda was adopted by the meeting with the adding of two additional presentations on the morning of the last day on the EU ReproTect project and the EU Cascade Network of Excellence.

OPENING OF THE MEETING

5. The Secretariat explained the background to the establishment of the VMG NA, and the decision by the 6th Meeting of the EDTA of the Test Guideline Programme in 2002 to start a 3rd VMG based on the great importance of, an urgent need for, relatively cheap and quick high-throughput screens and tests not requiring animals. The VMG NA was updated on the latest events of the EDTA and the WNT and that there is an ongoing discussion at the WNT of the exact roles of the EDTA and the three VMG's.

PRESENTATIONS

6. Masahiro Takeyoshi of CERI gave an update on the current status in Japan for ED non animal tests. The agonist part of the stably transfected Estrogen receptor (ER) transcriptional activation assay (STTA) has gone through validation and peer review and the antagonist part will be subjected to validation in 2008 under JaCVAM lead. (See table from presentation). He informed the meeting that an AR-EcoScreen assay was going through validation and that an SPSF will be submitted to the WNT. Laurence Musset (Secretariat) informed the meeting that the deadline for submission of SPSFs to the WNT20 was 31 January 2008. To not violate the guidance document No.34 rules that states that commercial tests cannot be developed into Test Guidelines unless a generic description and a set of performance standards are

provided, CERI have already asked Sumitomo Chemicals to make the cell line freely available at *e.g.*, the American Type Culture Collection.

7. Atsushi Ono (NIHS) gave an update with an aim for a HTPS assay

8. Hajime Koijima (JaCVAM) gave anUpdate on activities by the Japanese Center for the Validation of Alternative Methods of the MHLW (JaCVAM).

9. Since no representative of the Japanese Ministry of the Environment (MoE) was present to introduce the Detailed Review Paper (DRP) for Fish Receptor assays, the Secretariat gave a short update and asked for input from the meeting. A new session was for more in-depth analysis of the document was scheduled for the last day of the meeting. The Secretariat explained that the Japanese authors needed input on the most promising assays and whether we have any validated tests or can add any other substantial information to the draft DRP.

10. Miriam Jacobs (ECVAM) gave an update on the activities of ECVAM. See presentation. Ray Tice wondered whether cytotoxicity was evaluated in the antagonist assay and Alexius Freyberger explained that it had been mandatory. Ray further stated that different studies have used different limit concentrations, how do you deal with compounds that have been used at different concentrations? How do we handle the data in the future with different levels of activity? The chair Daniel Dietrich informed the meeting that some of these issues raised were already covered by the VMG NA4 meeting in Tokyo and meeting participants should read through the report before the planned discussions for the 2nd day.

11. Miriam described the latest developments of the DRP on Metabolism and that it will be submitted to the Joint Meeting (JM) in December 2007 for declassification. However, due to the high importance of aspects of metabolism for *in vitro* assays the topic will be a standing agenda item for future VMG NA meetings, which is in line with the WNT19 recommendation. Miriam further gave a short update on the most important issues that have been addressed since the last VMG NA meeting and the recommendations for short-, medium- and long-term prospects for metabolism assay developments.

12. Gary Timm (US EPA) gave a presentation of the validation status of the H295 Steroidogenesis assay that expresses all essential components of the steroidogenesis cycle and asked for input from the meeting for what endpoints should be applied, quantitative or qualitative? Expected to be completed by December 2008 when the peer review report will be made available. The validated cell line will be donated to the US National Institutes of Health cell line library.

13. Ray Tice (ICCVAM) presented the pre-validation and standardization work of the LumiCell™ assay. By using the outside wells instead of skipping them due to expected edge effects, they can double their testing of chemicals and will report in late 2008.

14. Shirlee Tan of the US-EPA gave a presentation over the phone on the latest developments for the FWA/CERI protocols for the human receptor ERα assays. The progress was noted and the assays will be validated in 2008 and a validation report is expected to be available by early 2009.

15. Pat Schmieder (US-EPA) gave an update on the work by the ED QSAR group that met before the VMG NA meeting. The primary purpose of the group is to promote exchange of information and increased global collaboration. The purpose of the group is not validation of QSAR's and the group meet and work independently of the VMG NA. The work by Japan and the USA on screening prioritisation and development of inventories with a purpose to prioritize chemicals for screening, generate hypotheses and to identify data gaps was presented. The latest development as to include metabolically active chemicals in the training sets. The USA's expert system for predicting estrogen hormone RBA for inert ingredients used

in food-use pesticides is nearing completion. During the next year it is anticipated that the USA will have the system documented in accordance with OECD's guidance for validating QSARs.

16. David Dix (US EPA) introduced the US EPA ToxCast programme. Problem to be solved: too many chemicals to be tested at a too high cost (www.epa.gov/comptox/toxcast). The Toxcast narrows down the present 90.000 chemicals that need additional assessment data to specific chemical groups (11.000 chemicals). ToxCast will function as a prioritizing tool for further testing across many endpoints (endocrine and non-endocrine) and it is based on pharmaceutical industry experience and drug discovery principles. ToxCast PHASE 1: ToxCast 320 is a subset of pesticides. In total 55 chemicals overlap between the ToxCast 320 and a list of approximately 75 compounds identified by the US-EPA screening program for Tier 1 screening in the US (note: these 75 chemicals were selected based on high exposure potential to humans and the environment only – these chemicals are not presumed *a priori* to have endocrine effects). 10.000 chemicals in >240 HTPS assays are expected to be screened until 2012. Signatures of toxicity in environmental chemicals will be evaluated. A total of 18 people are employed for the whole programme. A chemical library will be available on the website. ToxCast also collaborate with the toxicogenomic working committee at the OECD. The finished ToxCast Programme and derivative results will at the end be compared with existing data, and this will be done in cooperation with other EPA departments.

WEDNESDAY 14 NOVEMBER

Discussions on the STTA Assay

17. The Secretariat opened the meeting and explained that the goal should be to have the agonist STTA Test Guideline submitted to the WNT20 for adoption, which means that the VMG NA need to address all comments from member countries and to develop a performance standard for the assay. Given the short time line a revised draft should be submitted by the latest 2nd week of December to allow for expert commenting in member countries and give the Secretariat a realistic chance to submit the draft TG for approval at the WNT20 in early April 2008. The Secretariat also suggested merging the STTA subcommittee and the PBTG into one group, the STTA sub-committee (STTA-SC).

18. Miriam gave a presentation on the work of the STTA SC. The group agreed that the test should be used as a screen for prioritizing and not as a definite test and the assay response needs to be defined, not its classification properties. The terminology should be slightly changed and the response should be combined with a concentration to define: strong, moderate and negative activity at a given concentration. A number of rather difficult discussions of the assays performance were held. The group discussed why not testing should be done up to maximum solubility, however, since the test was not validated for this application testing up to maximum solubility would not be appropriate and a limit concentration should be set. The use of higher concentrations of DMSO (>1%) than what is outlined in the assay might lead to cytotoxicity and suppression (inactivation) of the reporter luciferase and therefore false-negatives. Ray outlined the three options; (i), use limit dose of xx mmolar; (ii), test until limit of solubility if you don't get a positive; and (iii), start somewhere and go up or down to a maximum concentration. Ray will provide some suggested text on this.

19. The other discussion items involved functional assay conditions such as mycoplasma infection monitoring, fold induction levels and responsive function and quality control.

Metabolism Working Group

20. Establishment of a Metabolism WG (Juliette Legler, Miriam Jacobs(coordinator), Christine Nellemann, Pat Schmieder, Alexius Freyberger, Dan Dietrich, Ray Tice, Gary Timm) that will check with ReproTect about S9-mix uses and other approaches. The group will report to the next VMG NA.

Discussions on PBTG

21. Gary Timm presented some options how performance-based Test Guidelines could be used and described some different scenarios. A lengthy discussion on the benefits and shortcomings of the different options followed and the Secretariat explained that the case with several test methods for the same endpoint are being developed and that me-too tests and performance standards for all of these will have to be developed is a new issue. There is one TG with detailed Performance Standards, and that is TG435, however there have never been any questions about how a TG435 me-too test should be judged, probably because there are no me-too developments for this endpoint. The Secretariat will consult with the OECD legal services if there may be legal problems with some of the options in respect of the Mutual Acceptance of data (MAD).

Discussion on SPSF's

22. Laurence Musset (Secretariat) introduced the SPSF issues and that 31 January 2008 is the deadline for submission of SPSF for the WNT20 meeting. We have already preliminary SOPSFs for the LumiCell, hERalpha and H295R assays that will be posted on the WNT WS. CERI will submit SPSFs for ERTA, ARTA and JaCVAM will hopefully submit an SPSF for the comet assay.

Table 1. Main ongoing projects and their validation status

Receptor Binding Assays				
hrERα	*Protocol 1.* The FWA assay protocol utilizes the Pan Vera hrERα full length ER. *Protocol 2.* CERI protocol utilizes the CERI-ERα, which contains the ligand binding domain of hrERα.	binding	Validation starting in early 2008 in 6 labs. SPSF submitted.	US lead international collaboration study
hrAR	Human recombinant AR assay. Ligand binding domain expressed in E. coli.	binding	Under development. Approximately 900 chemicals have been tested.	METI
	Human recombinant AR assay.	binding	Validation starting in 2008.	ECVAM Lead international collaboration study
hrTR	Human recombinant TR assay. Full-length expressed in E. coli. TRs α1 and β1 binding assays.	binding	Under development. Approximately 60 chemicals have been tested using both receptors.	METI
Transcriptional Activation Assays				
	HeLa-9903 cells with plasmids containing hERα cDNA driven by	Stable, ag/antag	The agonist assay draft TG will be proposed to WNT20 for adoption.	CERI/MHLW

ERα	SV40 promotor and luciferase reporter plasmid.		SPSF submitted. Antagonist assay validation will start in 2008.	
	HeLa-9903 cells: hERα/pcDNA3.1 receptor expressing plasmid and ERE-AUG-Luc+ reporter plasmid	Transient, ag	Validated under domestic multi-lab. using same test chemicals as hERa-HeLa-9903 cell line. Should be considered for (preliminary) Peer review.	CERI/MHLW
	MELN. MCF-7 cells with endogenous ERα + luciferase stably transfected	ag/antag	Validation in 2008.	EC/ECVAM
	ER-CALUX. T47 D (human breast cancer) cells with endogenous ERα + luciferase stably transfected	ag/antag	Validation planned for 2008.	EC/ECVAM
	LUMI cell, BG1 cells with endogenous ERα + luciferase stably transfected (XDS Inc)	binding	Validation will done by May 2008. Slightly delayed by the EC due to contract issues.	US lead international collaboration study
ERβ	HeLa, hERß/pcDNA3.1, ERE-AUG-Luc+	Transient, ag	Completed data collection for 250 compounds	CERI/MHLW
AR	CV-1 cells hAR/pcDNA3.1 receptor expressing plasmid and ARE-AUG-Luc+ reporter plasmid	Transient, ag/antag	Pre-validated and validated in Japan in 4 labs, with 5 chemicals. Should be considered for (preliminary) Peer review.	CERI/MHLW
	AR-Ecoscreen™ stable CHO clone	Stable, ag/antag	Validation report available in March 2008. SPSF will be submitted.	CERI/MHLW
	PALM. PC-3 (prostate adenocarcinoma) cells stably transfected with hAR and luciferase reporter gene	ag/antag	Validation in 2008.	EC/ECVAM
	CALUX. U2-OS (bone cell) cells stably transfected with hAR and luciferase reporter construct	ag/antag	Validation in 2008.	EC/ECVAM
TRβ	RXR co-transfected CHO cells are used	Transient, ag/antag	Under development, 150 chemicals tested so far.	MHLW
Aromatase & Steroidogenesis Assays				
	Microsomal aromatase		Validated, and the peer	

	assay, KGN cells		review report available in early 2008	
	H295R cell-based Steroidogenesis assay		Validation and peer review completed by December 2008. SPSF submitted.	US lead international collaboration study

THURSDAY 15 NOVEMBER

23. Christian Pelizzer of ECVAM presented of the ReproTect programme aiming at developing and optimising *in vitro* tests for reproductive toxicity endpoints. This is a EU project coordinated by ECVAM with a total budget 13 million €.

Continued Discussions on PBTG Issues

24. Steve Bradbury introduced the subject and concluded the previous days discussion and that MAD is probably the most important underlying requirement that may influence how we want to move this forward. With quickly emerging new strategies and new methods coming quickly we need to be adaptable without lowering the bar of acceptance and validation. The actual commenting round of new test methods is probably the most labour-some step after the actual validation. Steve introduced a set of three options based on Gary Timm's proposal.

> Option 1. *Status quo.* Keep the OECD adoption process as it is and develop individual Test Guidelines for all new test methods but the aim should be to have an expedited procedure for the adoption of me-too tests that meet the Performance Standards. No effects on MAD but will cause problems since the process is slow, and therefore tests could be outdated scientifically before they have been validated and adopted.
>
> Option 2. Develop PBTG for a specific endpoint with a separate compendium with detailed descriptions/SOP's for similar studies. There would be a streamlined way to have these "similar test methods" (me-too tests) accepted. One way could be to have a specific expert group (VMG NA, EDTA or a new Expert Group established for this particular purpose) that only examines these types of similar test methods and makes recommendations to the WNT for their adoption, or the specific expert group would make the decision whether the me-too test meet the requirements of the Performance Standard. Probably considerably quicker but may affect MAD.
>
> Option 3. PBTG approved by OECD but the "me-too-tests" could be adopted outside of the OECD procedures. Probably very vulnerable to MAD but would probably be a good procedure for some member countries that wants to keep up with the latest developments. This would give a self-certifying process by individual member countries.

25. The group discussed the different options and leaned towards option 1 and 2, however, with an emphasis of a quicker and smoother adoption process. The Secretariat explained that there are several projects were performance standards will be developed and ECVAM is setting up a special organisation to meet this challenge in the future. The Secretariat suggested that maybe another broader group with expertise in validation and performance standards should be involved in the development of performance standards and probably not the experts that have been involved in the validation of the test methods. The group generally agreed to the proposal, especially in the light of the fact that criteria for performance standard developments and how me-too tests can be used is an international issue that needs to be resolved relatively quickly. The group asked Laurence Musset to also consider discussions with legal services in

order to evaluate processes encompassing MAD yet greatly speeding up the process of validation and adoption without having too many layers of discussions and decisions.

26. The meeting suggested to establish a Performance Standards Issues Group (PSIG) (Secretariat, Gary Timm, Steve Bradbury, Alexius Freyberger, Dan Dietrich, Kate Willett) that would develop an issues paper for the WNT. The Secretariat explained that a consultation with OECD legal services regarding issues on MAD needs to be completed before an issues paper is developed. Therefore, it is probably more appropriate if the Secretariat develop a draft issue paper for review, discussion and comment by the PSIG before any recommendations provided to the WNT. Based on initial comments from the member countries the Secretariat may consider invitation one of the co-chairs of the VMG NA to the WNT meeting.

Discussion on the Fish *In Vitro* DRP

27. The meeting acknowledged the draft DRP but felt that a number of issues needs to be addressed in the DRP before it can be considered finalised and sent out for commenting to member countries. A Fish *In Vitro* Working Group comprising Dan Dietrich (coordination) Miriam Jacobs, Pat Schmieder, Jose Maria Savas, Susan Laws, Kate Willett and Les Touart will work on the document and submit an updated version to the Secretariat preferably by December 15, 2007 December. The Secretariat will share the document with the Japanese authors and will be asking them for revisions.

Update on EU CASCADE.

28. Lars-Arne Haldosen (Sweden) gave an update of the ongoing activities of the EU Network of Excellence

Follow-Up Work of the STTA

29. *PC10 and the wobbly base-line.* Yumi Akahori had a look at the raw data and it may not be so easy to resolve but need to get back to Japan before she can get the whole picture. Dan Dietrich and Alexius Freyberger emphasized that not interconnecting single values should be used to determine the PC10 but rather a curve should be optimized and integrated to lie within the data-points and therefore would cut the PC10-line only once.

Perfomance Standard for the agonist STTA.

30. Yumi Akahori gave a short presentation and suggested how to remove some outliers depending on the "zig-zag response". The feeling was that the PC10 was too close to the baseline. Yumi will check and put in some additional text. A compiled list of reference chemicals will be sent to Ray Tice from CERI and a selection of 10-15 reference chemicals will be used. Issues on true negatives came up, especially since the test has not been validated with maximum solubility, so it might be difficult to trust the data, why some negatives have to be cross-checked with other sources. Ray insisted that some of the test compounds have to be tested up to solubility.

31. Synopsis by the chair: Data needs to be gathered ASAP and Miriam will collate this but the Secretariat will have to give some justifications to the WNT why work is still ongoing and yet the draft Test Guideline is out for commenting. The final version with a complete list of chemicals will be available in February 2008 for the final review before the WNT20. Hopefully, no laboratory work needs to be done but this will follow the evaluation by Miriam Jacobs and Ray Tice.

Guidance on Chemical Selection for the STTA Antagonist Validation Study.

32. Hajime Kojima introduced the subject. The group decided that a chemical selection group comprising Hajime, Ray Tice and Miriam Jacobs will get together and clarify numbers of participating laboratories and the chemicals selected. The group will communicate with the Secretariat and the Secretariat will circulate this to the VMG NA for comments.

H295R Discussion

33. Gary Timm reintroduced the H295R issues and asked for input how should the data be interpreted? He will send his questions by e-mail to the group.

COMMITMENTS AND TIME FRAMES

34. The STTA-SC will develop a compilation of comments with expert's responses and a revised STTA TG including performance standards for the agonist assay within 2 weeks after the meeting for submission to the WNT for comments. The Secretariat will explain the issue with the chemical selection to the WNT in the accompanying letter.

35. The Fish *In Vitro* Working Group will report to the Secretariat preferably before 15 December and the Secretariat will communicate with the lead country, Japan.

36. The issues paper for the PBTG will be further developed by the Secretariat and circulated to the PSIG prior finalisation and submission to the WNT.

37. The VMG NA6 will be held in Paris in November 2008 and a suggestion for the 2009 meeting to be held in the US was presented and preliminary accepted.

38. A meeting report will be made available to participants for a short commenting period.

39. The VMG-NA thanked Patric Amcoff for his years of service, support and excellent advice and counsel to the validation group. All wished him well for the future.

ANNEX 1

List of Participants
(Only available for governmental representatives)

Patric.Amcoff@oecd.org
Paris, 19 December 2007

5TH MEETING OF THE VALIDATION MANAGEMENT GROUP FOR NON-ANIMAL TESTING (VMG-NA)
13-15 November 2007, ECVAM-DG JRC, Ispra Italy

DRAFT AGENDA (Version 1.2)

	Monday 12 November The meeting of the PBTG sub-committee and the STTA working group will take place at 15.45-18.00 at the Hotel Conca Azzurra, Via Alberto, 53, Ranco. Participants will meet in the lobby of the hotel for further information.	
Tuesday 13 November		
09h30-09h40	**Opening of the Meeting, Explanation of OECD Procedures** - There will be a brief explanation of the role of the members, the status of documents and other general procedures. Listing of Meeting documents.	
09h40-09h50	**Welcoming on behalf of the Hosting Institute (Thomas Hartung, ECVAM)**	
09h50-10h00	**Approval of the Draft Agenda**	
10h00-10h15	**Introduction of the Membership of the VMG-NA**	
10h15-10h30	**Introduction of the Special Activity on ED Testing by the Test Guidelines Programme** - There will be a brief summary of the history of the VMG's and the EDTA (Secretariat).	**Meeting documents 1 and 2**
10h30-10h45	**Update on Japanese activities** - Presentations of activities by CERI (Mashiro Takeyoshi)	**Meeting Documents 8, 9, 14**
10h45-11h15	**COFFEE/TEA BREAK**	
11h15-11h30	**Continued update on Japanese activities** - Presentations of activities by MHLW (Dr. Kojima or Dr. Ono) - The Draft Fish In Vitro Receptor DRP (Secretariat)	**Meeting Documents 8, 9, 14**
11h30-12h00	**Update on ECVAM activities** - Presentation by ECVAM on validation status of ED *in vitro* ER and AR TA assays (Miriam Jacobs) - Discussions	**Meeting document 9**

12h00-12h30	**Status of the DRP on Metabolism** - Presentation by Miriam Jacobs (ECVAM)	**Meeting Document 7**
12h30-13h45	**LUNCH BREAK**	
13h45-14h15	**The H295R Validation Study Plan** - Presentation by Gary Timm (US EPA) - Discussions	**Meeting Document 12**
14h15-15h00	**The LumiCell Pre-validation Report** - Presentation by Ray Tice (US NICEATM) - Discussions	**Meeting Document 15**
15h00-15h30	**COFFEE/TEA BREAK**	
15h30-16h30	**The Validation Plan for the Human Recombinant ER-Binding Assay** - Presentation by Shirlee Tan (US EPA) - Discussions	**Meeting Document 12**
16h30-17h10	**Presentation on the US EPA ToxCast Programme** - Presentation by US EPA (David Dix)	**Meeting Document 15**
17h10-17h30	**Presentation on Progress of the QSAR Group** - Presentation by Pat Schmieder (US EPA)	
17h30	**ADJOURN FOR THE DAY**	
Wednesday 14th November		
09h30-09h40	**Review Day 1 and Objectives for Day 2**	
09h40-10h30	**Over view of the Work of the Stably Transfected ER Transcriptional Activation Assay Sub-Committee (STTA SC)** - The work and proposed strategy of the STTA SC will be presented by Masahiro Takeyoshi (CERI) - Discussions	**Room Documents 2 and 3**
10h30-11h00	**COFFEE/TEA BREAK**	
11h00-12h30	**Continued Discussions on the STTA SC**	
12h30-13h45	**LUNCH BREAK**	

13h45-14h45	**Over view of the Work of the Performance-Based Test Guidelines Working Group (PBTG WG)** - The work and proposed strategy of the PBTG WG will be presented by Miriam Jacobs (ECVAM) - Performance Standards at the OECD (Secretariat) - Discussions	**Room Documents 1, 2 and 3**
14h45-15h15	**COFFEE/TEA BREAK**	
15h15-16h00	**Continued Discussions on the PBTG WG**	
16h00-17h30	**Standard Project Submission Forms** - The Secretariat will introduce the topic - Discussions	**Meeting Documents 9, 10, 11**
Thursday 15th November		
09h30-09h40	**Review Day 2 and Objectives for Day 3**	
09h40-11h00	**Continued Discussions on the STTA and PBTG Activities and ways forward** - Discussions - Work plans, establishment of sub-groups, dead lines, etc,	
11h00-11h30	COFFEE/TEA BREAK	
11h30-12h00	**Any Other Business**	
12h00-13h00	**Concluding Discussions Time Frames and Commitments for Activities**	
13h00	**MEETING ADJOURNED**	

Meeting Documents

Documents are available on the protected website:
http://webdomino1.oecd.org/comnet/env/tf-edta.nsf?OpenDatabase
User name <<more>>, Pass word <<estrogen>>; and then go to VMG NA5

Meeting Document #1	**Meeting Report from EDTA 10, 27-28 March, 2007, Paris [ENV/JM/TG/EDTA/M(2007)3/REV 1]**
Meeting Document #2	**Meeting Report from the 4th Meeting of the VMG-NA, December 2006 [ENV/JM/TG/EDTA/M(2006)4]**

Meeting Document #3	Guidance Document No. 34 on the Validation and International Acceptance of New or Upgraded Test Methods for Hazard Assessments
Meeting Document #4	Test Guidelines 435 on In Vitro Membrane Test for Corrosivity Testing
Meeting Document #5	Test Guidelines 430 "EPISKIN"
Meeting Document #6	Test Guidelines 431 "TER"
Meeting Document #7	Draft Detailed Review Paper for the Use of Metabolising Systems for In Vitro Testing of Endocrine Disrupters.
Meeting Document #8	Draft Detailed Review Paper for Fish In Vitro Receptor Binding Assays
Meeting Document #9	SPSF for Stably Transfected Transcriptional Activation Assay for the Detection of Estrogen Receptor Agonists and Antagonists
Meeting Document #10	SPSF for Human Recombinant Estrogen Receptor Alpha Binding Assays
Meeting Document #11	SPSF for H295R Cell-based Steroidogenesis Assay
Meeting Document #12	Update on US EPA's In Vitro Method Developmental Activities
Meeting Document #13	Status report on the human recombinant ERα-binding pre-validation effort (2007)
Meeting Document #14	Update Validation Status of Non-animal Testing in Japan by CERI, METI, NIHS. MHLW and MOE.
Meeting Document #15	Update on US-EPA's VMG NA In Vitro Activities
ROOM DOCUMENTS	
Room Document #1	Minutes of the Performance Based Test Guidelines Task Group (PBTG TG) Teleconference, Tuesday 23 October 2007.
Room Document #2	Minutes of the STTA sub-committee telephone conference, Tuesday 23 October 2007
Room Document #3	*Documents for the STTA sub-committee:* 3.1: Letter PA.2007.15_STTA PRP_12 September_ 07 3.2: Letter PA.2007.13 STTA PRP and Comments 3.3: Validation Report STTA 3.4: STTA Test Guidelines draft 3.5: JM Letter by Rob Visser 3.6: WNT19-JM-Declass-PRP-STTA

OECD TEST GUIDELINES PROGRAMME

Standard Project Submission Form

If you require further information please contact the OECD Secretariat
Return completed forms to:
env.tgcontact@oecd.org

PROJECT TITLE

Stably transfected Transcriptional Activation (TA) assay for detection of anti-estrogenic activity of chemicals

SUBMITTED BY (Country / European Commission / Secretariat)

Yumiko Nomura and Ayumi Kodama

DATE OF SUBMISSION TO THE SECRETARIAT

January 2008

DETAILS OF LEAD COUNTRY/CONSORTIUM

Country /Organisation:	Japan
Agency/ministry/Other:	Ministry of Health, Labour and Welfare (MHLW), Japan and Ministry of Economy, Trade and Industry (METI), Japan
Mail Address:	Kasumigaseki 1-2-2, Chiyoda-ku Tokyo, Japan and Kasumigaseki 1-3-1, Chiyoda-ku Tokyo, Japan
Phone/fax:	81-3-3595-2298/81-3-3593-8913 and 81-3-3501-0080/81-3-3580-6347
Email:	nomura-yumiko@mhlw.go.jp and kodama-ayumi@meti.go.jp

PROJECT OUTCOMES

☒ New Test Guideline	☐ Guidance document
☐ Revised Test Guideline	☐ Detailed Review Paper
☐ Deletion of an existing Test Guideline	☐ Other, please specify below

PROPOSED WORK PLAN and RESOURCE NEEDS:

1. Draft workplan for development of the proposal, including any need to establish Ad Hoc Expert Group and mode of meetings (face-to-face, teleconference; electronic discussion group). Indicate key milestones, including first and subsequent drafts of documents and timing of meetings.

The validation study for a method using estrogen responsive stable cell line using HeLa9903 cell line to detect anti-estrogenic activity will be completed by early 2009 and its validation report and its draft guideline will be available by middle 2009. At the same time, the scientific peer review will be initiated and the report from the scientific peer review will be prepared until late 2009.

2. Will additional information, including generation or collection of data, be required? If yes, please describe the anticipated process and timelines.

The multi-lab validation study using 10-12 coded chemicals including 3-4 laboratories under the lead of JaCVAM (Japanese Center for the Validation of Alternatives) will be initiated in early 2008. The participation from Japanese and European laboratories is planed.

3. Indicate the estimated overall resource need (time/money) for member country / consortium and Secretariat

- Time: At least, six weeks of peer review process 3-4 weeks for the consultant to prepare and finalize the peer review report.
- Money: At least, 20,000-EUR would be needed if employ independent consultant for the peer review process.

4. Is this proposal intended to replace an existing Test Guideline or lead to the deletion of an existing Test Guideline?

No